Team iGEM UFSCar-Brasil

Final Safety Form – iGEM 2015

Do you plan to experiment with any other organisms, besides your chassis?

Our project involves the use of Escherichia coli DH5 alfa strain as the main chassi in our experiments of limonene synthase expression. Our genic circuit comprises 3 modules: the expression of limonene synthase, chaperon expression and the assembly of a Kill Switch. The main experiments consist in purification and quantification of limonene molecules. Finishing the assembly of our genic circuit, we plan (further, not for Giant Jamboree presentation) to use Lactobacillus sp. as chassi. In this way, will avoid some issues that are found using E. coli, like inconvenient smell and its pathogenicity.

How will your project work?

Our project aims to develop bacteria that is capable of producing suitable limonene, a volatile terpen that helps in the prevention of mosquitoes transmittable diseases like dengue and malaria. In this way, a repellent with a long life-span will be produced using E. coli in a solution of Polyethylene glycol (PEG) as a final product. The bacteria will be in a smoldering state, with contraption of its volume, also known as plasmolysis. When applied to skin, the sweat will dilute PEG, leading to an osmotic stress that will promote the activation of a promoter (UspA) that regulates limonene synthase. Once expressed, the enzyme will use geranyl-pyrophosphate as substrate to produce limonene. Besides, the stressing situation promotes the co-expression of different classes of chaperones, which are expected to remodel the enzyme correctly. The bacteria will then be able to express chaperones of classes ClpB, DnaK and IbpA/IbpB. The developed engineered bacteria also feature a kill switch system based on E. coli operon znuABC, preventing the dissemination of bacteria to the environment. Other kill switch systems are being considered, like BaRNAse that will degrade all the bacterial RNA, and Killer Red that promotes the production of reactive oxygen species.

What risks does your project pose at the laboratory stage? What actions are you taking to reduce those risks?

Our project does not pose any risks at the laboratory stage, because we are only using parts from the white list. We also follow good laboratory manners to reduce any possible risks.

What risks might your project pose, if it were fully developed into a real product that real people could use? What future work might you do to reduce those risks?

Considering that our real final product involves a genetically modified organism that is applied to human skin, it could pose some risks to humans and environment. In order to avoid these risks, we will develop a kill switch for our bacteria that will destroy its genetic material based on the availability of zinc. Furthermore, all of our engineered genetic circuit will be transferred to the bacterial genome by the action of transposases and recombinases. Thus, this genomic integration would reduce the risk of genetic material transfer to other microorganisms when compared to the use of plasmidial form.

Any further comments about your project:

Our project presents a big appeal in Brazil, where the dengue is a disease that each year grows in great part of the states. In 2015, São Carlos city was in an epidemic state and even members of our team were affected. The production of limonene by the engineered bacteria represents a strategic method to fight the mosquito with a simple appliance of a repellent cream (or spray), avoiding its bite. Besides, limonene presents much lower toxicity compared to other commercial repellents.

Comments about this form: Is it easy or difficult to use? Are the questions confusing?

Is was a clear form and easy to understand.